American Journal of Hematology 63:46–53 (2000)

Donor Cell Leukemia: Report of a Case Occurring 11 Years After Allogeneic Bone Marrow Transplantation and Review of the Literature

Linda D. Cooley,[1] David A. Sears,[2]* Mark M. Udden,[2] Wilbur R. Harrison,[1] and Kelty R. Baker[2]

[1]Department of Pathology and Laboratory Medicine, The University of Texas Medical School, Houston, Texas
[2]Department of Medicine, Hematology Section, Baylor College of Medicine and Ben Taub General Hospital, Houston, Texas

We report the case of a man with chronic myelocytic leukemia (CML) and a 46,XY,t(5;9;22) karyotype who developed acute myelocytic leukemia (AML) with a 45,X,t(8;21) karyotype 11 years after bone marrow transplantation (BMT) from his HLA-matched sister. Fluorescent in situ hybridization (FISH) studies and molecular analysis using short tandem repeat (STR) sequences proved the new leukemia to be of donor cell origin. Donor cell leukemia (DCL) after BMT is rare. Our review of the literature found 15 cases following BMT for leukemia and 2 cases after BMT for benign hematological disorders. In fewer than half the reported cases were molecular studies available to confirm the cytogenetic evidence for DCL, and the longest previously reported interval between BMT and DCL was 6 years. Am. J. Hematol. 63:46–53, 2000. © 2000 Wiley-Liss, Inc.

Key words: bone marrow transplantation; leukemia; recurrence

INTRODUCTION

Following BMT, patients are at substantially increased risk for the development of second cancers, both solid tumors and hematological malignancies [1–7]. Factors increasing this risk include an HLA-nonidentical donor, T-cell depletion of the donor marrow, treatment with total body irradiation (TBI) in the conditioning regimen, and treatment with anti-thymocyte globulin (ATG) or other anti-T cell antibodies for graft versus host disease (GVHD) [8].

The most frequent cause of treatment failure after allogeneic BMT for leukemia is relapse of the underlying host leukemia [9]. However, rare cases have been reported in which cytogenetic analysis, alone or in combination with molecular analysis, has indicated that the new leukemia originated in donor cells. On the basis of cytogenetic studies of 54 relapses in sex-mismatched BMTs, Boyd et al. suggested that DCL accounted for approximately 5% of relapses [10]. We report here a case of DCL with unusual features and review previously reported cases.

CASE REPORT

The patient was a 25 year old Hispanic man who was admitted to Ben Taub General Hospital in February 1982 with a 4-month history of 100-pound weight loss and recent symptoms of early satiety, dysphagia, and abdominal fullness and tenderness. Physical examination was unremarkable except for massive splenomegaly; the spleen extended to the pelvic brim and well to the right of the umbilicus. Laboratory values were as follows: hematocrit 20%, platelet count 128,000/μl, and white blood cell (WBC) count 56,000/μl. The blood smear showed a range of precursor and mature neutrophils, 3% eosinophils, 3% basophils, and occasional nucleated red blood cells. The leukocyte alkaline phosphastase score was 0. The bone marrow was hypercellular with a large increase in granulocyte precursors at all maturation stages. Cytogenetic studies showed a complex translocation, t(5;9;22), resulting in the Philadelphia chromosome. The diagnosis of CML was made, and treatment with busulfan was begun. The patient responded to therapy with a decrease in symptoms and improvement in blood counts. Follow-up was irregular, and in February 1985, when he

*Correspondence to: David A. Sears, M.D., Department of Medicine, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030. E-mail: dsears@bcm.tmc.edu

Received for publication 18 March 1999; Accepted 6 October 1999

presented in chronic phase relapse, treatment with hydroxyurea was begun.

On March 31, 1986, he experienced the sudden onset of severe left upper quadrant abdominal pain, and physical examination revealed a loud friction rub and palpable thrill over the spleen. Laboratory values included hematocrit 30%, platelet count 158,000/μl, and WBC count 23,200/μl. The diagnosis of splenic infarction was confirmed by ultrasound and computerized tomographic studies. Because of persistent pain and fever, splenectomy was performed. The organ measured 46 × 30 × 16 cm and weighed 33 pounds.

Pathological examination showed numerous infarctions and a small amount of extramedullary hematopoiesis. The patient's platelet count, and to a lesser degree, his WBC count, increased markedly, requiring higher doses of hydroxyurea. BMT was planned.

One of the patient's three sisters was found to be identical to the patient at 5 HLA loci; one of the patient's DR loci was indeterminable. A mixed lymphocyte culture was unreactive. The patient was given a conditioning regimen of cyclophosphamide, 60 mg/kg/day, intravenously for two consecutive days followed by TBI, 1,200 cGy, delivered in 6 fractions over three days. On May 22, 1986, one day after completion of TBI, he was infused with 3×10^{10} nucleated bone marrow cells from his sister. Methotrexate and cyclosporine were administered for GVHD prophylaxis. By the 18th day after transplantation, rising blood counts indicated engraftment. On the 25th day the bone marrow was 30% cellular with orderly hematopoiesis and a 46,XX karyotype. He was discharged from the hospital.

He did well for three months until September 7, 1986, when he had the sudden onset of abdominal pain and vomiting. The diagnosis of small bowel obstruction was made. He underwent laparotomy for the lysis of adhesions and reduction of obstruction. One day later a second laparotomy was performed for the resection of 52 cm of necrotic small bowel followed by jejunojejunostomy.

The patient's methotrexate and cyclosporine were stopped at the time of his bowel obstruction, and he did well except for minor skin changes thought to reflect GVHD. A bone marrow examination in March 1987 was normal and had a 46,XX karyotype.

In November 1987, 1 1/2 years after BMT, the patient was hospitalized for 7 days with disseminated herpes zoster infection of the skin which responded to intravenous acyclovir. The patient did well and was not seen after November 1991 until August 1997, 11 years after BMT, when he was admitted to the hospital with pain in the left buttock. A large perirectal abscess was drained, and he was found to be severely anemic and thrombocytopenic. The white blood cell count was 75,000/μl, and the blood smear showed 90% blast cells. The bone marrow was also dominated by blast cells similar to those in the blood. These were cells of medium-to-large size, some of which contained prominent nucleoli, folded or cleft nuclei, and granular cytoplasm with rare Auer rods. Most of the blast cells stained with Sudan black. About 10% stained strongly positive and an additional 20–30% weakly positive for nonspecific esterase. The periodic acid Schiff stain was negative. Flow cytometry for surface markers showed positivity for CD 34, 33, 11c, and 56 and for HLA-DR, suggesting immature myeloid lineage. Cytogenetic studies are discussed below. The diagnosis of acute myelomonocytic leukemia was made. Cultures of the perirectal abscess grew *Escherichia coli*, and the patient was treated with antibiotics. On August 30, 1997, anti-leukemic chemotherapy was initiated with a 7-day infusion of cytarabine and 3 daily injections of daunarubicin. The patient remained febrile and became severely leukopenic. A subsequent blood culture was positive for *Pseudomonas aeruginosa.* The patient developed progressive acidosis, renal insufficiency, disseminated intravascular coagulation, and respiratory failure, and died on September 16, 1997.

The autopsy showed disseminated candidiasis and leukemic infiltrates in the lungs, liver, lymph nodes, kidneys, and gastrointestinal tract.

METHODS

Cytogenetics

Bone marrow and blood samples were cultured for 24 and 48 hr in RPMI 1640 medium supplemented with 15% fetal bovine serum or 10% fetal bovine serum and 10% conditioned medium (Boehringer Mannheim). Cells were harvested, and slides were made and GTG banded using standard cytogenetic methods. Fifteen to 20 cells were completely analyzed with at least 2 cells karyotyped from each study. Karyotypes are described using ISCN 1995 [11].

FISH

Slides for FISH were prepared following standard cytogenetic procedures and stored at 37°C. until use. The sex chromosome complement was determined by scoring 100 interphase nuclei hybridized to a dual color α satellite X (DXZ1) and satellite III Y (DYZ1) probe (Vysis Inc.). The bcr/abl rearrangement was determined by scoring 100 interphase nuclei hybridized to the Vysis LSI bcr/abl probe. Slides made from the blood of a normal male were hybridized and scored in an identical manner for controls. Both probes were used following the manufacturer's protocols.

STR Sequence Marker Studies

DNA was extracted (Puregene kit, Gentra Systems Inc.) and evaluated with 3 primer sets (Map Pairs, Research Genentics Inc.) for STR markers on chromosomes

TABLE I. Cytogenetic and FISH Results*

Study date	Result	Sample
2/25/82	46,XY,t(5;9;22)(q13;q34;q11.2)[11]	BM
6/16/86	46,XX[30]	BM
3/9/87	46,XX[30]	BM
8/26/97	45,X,t(8;21)[12]/46,XX[3]	PB
8/26/97	X[91]/XX[9]/XY[0] by FISH	PB
8/26/97	bcr/abl negative [100] by FISH	PB

*Number of cells analyzed in brackets. BM, marrow; PB, blood.

8, 10, and 12. Polymerase chain reactions were performed using published protocols [12], and gels were stained using the GELCODE system (Pierce Inc.).

RESULTS

Cytogenetics

The results of cytogenetic and FISH studies are shown in Table I. The first bone marrow cytogenetic study, performed at the time of diagnosis of CML, showed a 3-way translocation involving chromosomes 5, 9, and 22, i.e., t(5;9;22)(q13;q34;q11.2) (Fig. 1). Bone marrow cytogenetic studies in 1986 and 1987 after BMT showed only normal female cells. No further cytogenetic studies were performed until the time of the patient's new leukemia in 1997 when an unstimulated peripheral blood sample was analyzed. This study showed a 45,X,t(8;21)(q22;q22) karyotype in 12 cells (Fig. 2) and a 46,XX karyotype in 3 cells.

FISH

Hybridization of the patient's 1997 blood sample described above with the dual color X/Y probe showed 9% of cells to have two X signals and 91% of cells to have a single X signal. No cells showed a Y signal. A normal male control showed hybridization signals for 1 X chromosome and 1 Y chromosome for all the nuclei scored.

Hybridization of the same patient blood sample with the bcr/abl probe showed 102 of 106 cells with no fusion signal. This was consistent with the 3% fusion signal positive cells in the control.

STR Sequence Markers

To determine the origin of the cells with the 45,X,t(8;21) karyotype, DNA was extracted from blood obtained from the marrow donor, the leukemic blood sample from the patient, and the patient's kidney obtained at autopsy. The DNA was used to perform STR studies.

Figure 3 shows the genotyping results from the patient's kidney and blood, and from the donor's blood. The chromosome 10 marker was fully informative, showing that the patient's and donor's blood had identical alleles that were different from both alleles in the patient's kidney. The chromosome 8 and chromosome 12 markers were partially infomative, showing one allelic difference.

DISCUSSION

DCL, though rare, is an intriguing entity. It has been difficult to verify and has sparked debate about the possible mechanisms whereby it arises. From the time of the first reported case of DCL in 1971 [13] until 1985, routine cytogenetic analysis with quinacrine banding and Y body detection by fluorescent microscopy were the only methods utilized to document the origin of the second leukemia. Since 1985, more reported studies have utilized molecular and molecular cytogenetic methods in combination with routine cytogenetic analysis to differentiate DCL from host leukemia relapse. The newer methodologies have shown that cytogenetic analysis alone may suggest DCL when, in fact, the leukemia is due to host leukemia relapse.

In our patient, a cytogenetic study at the time of the initial diagnosis of CML showed the presence of a clonal chromosome abnormality, a three-way translocation that resulted in the Philadelphia chromosome. Cytogenetic studies after BMT confirmed engraftment of the donor marrow with a normal female karyotype. When the patient again developed leukemia 11 years after BMT, the karyotype showed a new clonal chromosome abnormality, t(8;21), as well as loss of one sex chromosome in the majority of cells and a 46,XX karyotype in the remaining cells. The finding of a new and unrelated clone indicated that the patient had a "new" leukemia, and the FISH studies with bcr/abl probes confirmed that the previously observed t(5;9;22) was not present. Although it seemed apparent that the patient had a new leukemia, the cell of origin could not be assumed. FISH studies using X and Y probes confirmed the cytogenetic findings of a single X chromosome in the majority of cells, two X chromosomes in a minority of cells, and no cells with a Y chromosome. The absence of the Y chromosome did not rule out the possibility that the new clone arose from a male cell since the t(8;21) is frequently associated with loss of the Y chromosome in male patients [14]. Hybridization of short tandem repeat sequence markers from three different chromosomes to DNA from the donor blood, the leukemic host blood, and the host kidney gave unequivocal evidence that the leukemic blood DNA matched the donor blood DNA, and not the patient's DNA, thus confirming DCL.

Seventeen cases of DCL have previously been reported after BMT for leukemia (15 cases) or for benign diseases (2 cases) [13,15–35]. These cases and the case reported here are summarized in Table II.

Of these 18 cases of DCL, 10 patients were female and

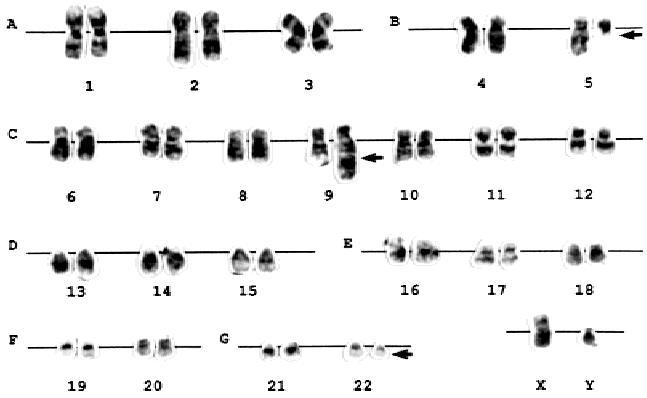

Fig. 1. Karyotype. At diagnosis of CML with t(5;9;22)(q13;q34;q11.2). Arrows point to abnormal chromosomes.

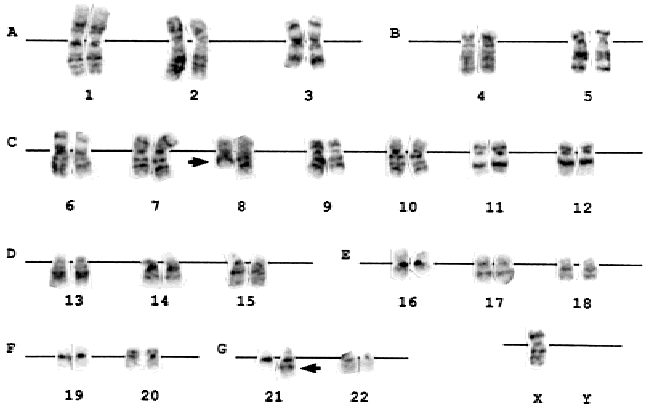

Fig. 2. Karyotype. Donor cell leukemia with 45,X,t(8;21)(q22;q22). Arrows point to abnormal chromosomes.

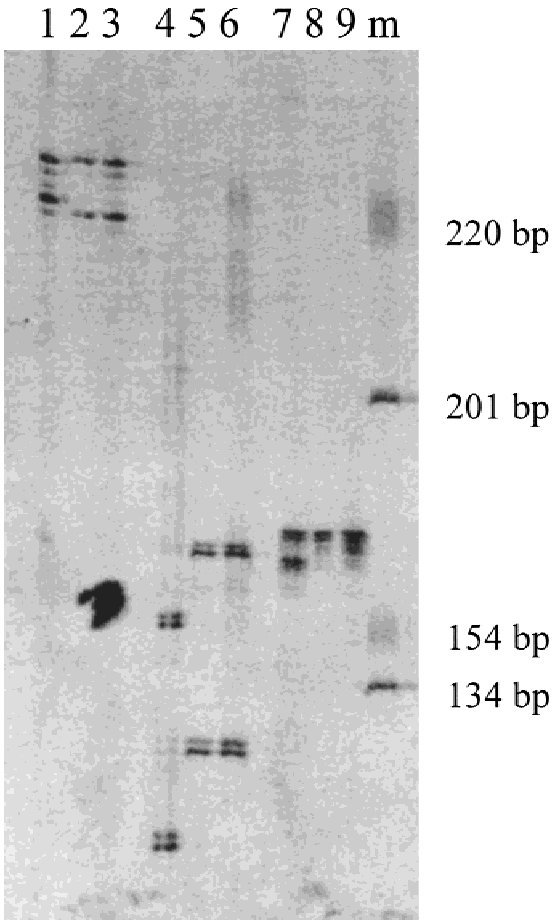

Fig. 3. STR sequence marker gel. Lanes 1, 4, and 7 contain DNA from patient kidney tissue. Lanes 2, 5, and 8 contain DNA from patient leukemic blood. Lanes 3, 6, and 9 contain DNA from donor blood. Chromosome 12 (D12S391) marker in lanes 1, 2, and 3; chromosome 10 (D10S2325) marker in lanes 4, 5, and 6; and chromosome 8 (D8S1132) in lanes 7, 8, and 9. Lane (m) contains DNA size marker.

8 were male. Ages ranged from 3 to 43 years. Nine patients were 16 years of age or younger, and 9 were over 16. BMT was for acute lymphocytic leukemia (ALL) in 7, acute myelocytic leukemia (AML) in 4, CML in 5, aplastic anemia in 1, and thalassemia in 1. The donor cell leukemia was the same cell type as the pre-BMT leukemia in 10 of the 16 leukemic patients. Of the other 6 leukemic patients, 5 underwent BMT for CML. Three of these (cases 6, 7, and 12) relapsed with ALL in donor cells; 2 (cases 6 and 7) had undergone transformation to ALL before BMT, but with treatment had reverted to chronic phase (case 6) or remission (case 7). One CML patient (case 15) developed AML in donor cells after an antecedent myelodysplastic syndrome. Our patient (case 16) developed an apparent de novo AML. The final patient (case 13) underwent BMT for AML and developed Burkitt-type ALL in donor cells. Of the 2 patients transplanted for benign disease, one developed ALL and the other AML.

Fifteen of the 18 DCL patients received marrow from sex-mismatched donors. The leukemic cells in 13 of the 15 had the sex chromosomes of the donor. In one of the remaining 2, the recurrent leukemia was said to involve both host and donor cells (case 3), and in the other (case 4) the 45,X karyotype was thought to reflect loss of an X chromosome from donor cells based on the results of quinacrine fluorescent banding studies. In the three patients who had same-sex donors (cases 5, 8, and 13), the donor cell origin of the leukemia was demonstrated by quinacrine banding in one (case 5) and by molecular studies in the other two.

Identification of the cell of origin of the recurrent leukemia depended on cytogenetic analyses, with or without quinacrine fluorescent studies, in 11 of the reported cases. Most often, identification of the sex chromosomes of a sex-mismatched donor provided the major evidence. Such evidence may not be trustworthy. Cytogenetic study of leukemic samples depends on spontaneous cell

TABLE II. Reported Cases of Donor Cell Leukemia*

Pt no.	Ref	Age sex	Donor	Conditioning Rx	GVHD Rx	Time from BMT→DCL	Type of leukemia		Cytogenetics		Studies at Relapse	
							Original	Relapse	Pre-BMT	Post-BMT with DCL	Molecular	Other
BMT for leukemia												
1	13	16F	Brother	TBI	MTX	2 mo	ALL	ALL	None	46,XY[240]		autoradiography, Q-stain
2	15	7F	Brother	TBI	MTX	4.5 mo	ALL	ALL	46,XX[94]	46,XY[359]		Fluorescent interphase Y-body
3	16	4F	Brother	Ctx	Ctx	21 mo	AML	AML	46,XX[94]/ 47,XX,+F[7]	46,XX[10]/47,XX,+F[3]/ 46,XY[47]/47,XY,+F[5]		Fluorescent interphase Y-body
4	17	22M	Sister	Ctx	MTX, Ctx	34 mo	AML	AML	46,XY[12]	45,X,t(8;21)[13]/46,XX[24]		Q-stain for Y & heteromorphisms
5	18	12M	Brother	TBI, Ctx	MTX	20 mo	ALL	ALL	46,XY#	46,XY#		Q & chromomycin A3 stains for heteromorphisms
6	19, 29, 31	21F	Brother	TBI, Ctx	CsA, Steroids	6 mo	CML	ALL	46,XX,t(9;22)[8]	46,XY,t(9;22)[40]	Microsatellite repeats by pcr	
7	21	9M	Sister	TBI, Ctx		21 mo	CML	ALL	46,XY,t(9;22)[7]	46,XX[44]/46,XX,inv(9)[6]		Q-stain for Y-body
8	20	25F	Sister	TBI, Ctx, MTX IT	MTX, ATG, Steroids	6 yr	ALL/ AML	AML	None	47,XX,5p−,7p+,−8,14q+, −16,−16,−22,−22,+6mar[6]/ 46,XX[4]	RFLP analysis	
9	24	22F	Brother	TBI, Ctx, MTX	MTX	4 yr	T-ALL	T-ALL	46,XX	46,XY,del(6)(q23q25)[2]/ 45,X,−4,6q,+8,−15,i(21q), +mar[3]/46,XY[15]		
10	25	3F	Brother	TBI, Ctx	Mtx, Steroids	2 yr	T-ALL	B-ALL	46,XX[12]	46,XY[101]	RFLP analysis	
11	22, 23, 26	12F	Brother	TBI, Ctx	CsA	3 yr	ALL	ALL	46,XX[20]	46,XY[40]/47,XY,+8[12]		
12	28	30M	Sister	TBI, Ctx, splenic irrad	MTX, CsA, Steroids	1 yr	CML	ALL	46,XY,t(9;22)	46,XX[10]		
13	33	37F	Sister	TBI, Ctx	MTX, CsA, Steroids, Thalidomide	3 yr	AML	B-ALL	46,XX[20]	46,XX,t(14;18)[20]	VNTR analysis at D10S28, AMP/FLP	
14	32	16F	Brother	TBI, Ctx, Ara-C	Steroids	1 yr	AML/ ALL	ALL	None	46,XY[45]		ISH with Yp probe
15	34, 35	43M	Sister	TBI, Ctx, Ara-C		3 yr	CML	AML	46,XY,t(9;22)	45,XX,−7[25]		FISH for chromosome 7
16	C	29M	Sister	TBI, Ctx	CsA	11 yr	CML	AML	46,XY,t(5;9;22) [11]	45,X,t(8;21)[12]/46,XX[3]	STR analysis	FISH for X and Y, FISH for bcr/abl
BMT for benign disease												
17	27	19M	Female cousin	Ctx, ATG	CsA, MTX	9 mo	Aplastic Anemia	AMoL	46,XY[15]	46,XXt(9;11)[20]	Microsatellite repeats by pcr	
18	30	5M	Sister	Ctx, Busulfan	CsA, MTX	5 yr	Thalassemia	ALL	None	46,XX	RFLP analysis	FISH for X and Y

*Abbreviations: TBI, total body irradiation; Ctx, cytoxan; MTX, methotrexate; ATG, antithymoctye globulin; IT, intrathecal; Ara-C, cytarabine; CsA, cyclosprin-A; AML, acute myelocytic leukemia; ALL, acute lymhocytic leukemia; CML, chronic myelocytic leukemia; AMoL, acute monocytic leukemia; Q-stain, banding with quinacrine using fluorescence; F, F group chromosome 19 or 20; t, translocation; inv, inversion; del, deletion; mar, marker; i, isochromosome; p, short arm of chromosome; q, long arm of chromosome; RFLP, restriction fragment length polymorphism analysis; AMP/FLP, amplified fragment length polymorphism analysis; STR, short tandem repeats sequence analysis; pcr, polymerase chain reaction; VNTR, variable number tandem repeats; #, chromosome analysis on male patient and male donor performed, but actual karyotype not given; C, current case; number of cells analyzed.

division. If the leukemic cells are nondividing or slowly dividing, they may not be captured in metaphase, and thus the cytogenetic study may reflect only the donor cell population [36–38]. Alternatively, the methods used to prepare or capture metaphases may select against a leukemic clone [37]. Furthermore, sex chromosomes may be lost from leukemic clones and transfected from one cell line to the other [39–41].

The more recently employed molecular studies of DNA polymorphisms can identify the cell of origin with confidence [42–48]. Such studies were employed in seven of the cases of DCL summarized in Table II (cases 6, 8, 10, 13, and 16–18).

The incidence of recurrent leukemia in host cells diminishes with time and is very rare after six years [2,49]. On the other hand, donor cell leukemia tends to occur late. Only four of the 18 cases of DCL presented within the first year after BMT. The other 14 occurred 1–11 years after BMT. The 11-year interval in our patient is the longest reported. The longer the interval after BMT, the more likely it is that the relapse involves donor cells. It is possible that most late relapses occur in donor cells.

DCL has stimulated interest, despite its rarity, because of the expectation that it might provide insights into the etiology and mechanisms of leukemogenesis. Several hypotheses have been offered to explain how DCL might arise.

Occult leukemia in the donor: Although leukemia was inadvertently transmitted by BMT in one reported case [50], none of the marrow donors in the cases reviewed here subsequently developed leukemia.

Transfer of oncogenic material from host to donor cells: Conditioning therapy, particularly TBI, might release viral or nonviral oncogenic material from damaged leukemic cells and transfect the donor cells administered shortly thereafter [13,15]. Fusion of residual host cells with donor cells is another mechanism whereby host material could be transferred to donor cells [29]. In early reports of DCL (cases 1 and 2 in Table II), bone marrow examinations and cytogenetic studies soon after BMT showed severe radiation damage to chromosomes of host cells [13,15]. This led the authors to add chemotherapy to their conditioning regimen [15]. In another patient, who received no TBI (case 3), a chimeric state existed after BMT, and the recurrent leukemia affected both host and donor cells similarly [16]. In case 6 the t(9;22), present in host cells at the time of BMT, was found in donor cells at relapse 6 months later (19, 29, 31). In several other cases the relapse in donor cells was identical to the original leukemia, a finding that would be compatible with the transfer of oncogenic material from host to donor cells. Witherspoon and co-workers have pointed out that this "single hit" hypothesis of leukemogenesis becomes less tenable as the interval from BMT to recurrent leukemia becomes longer [20]. Additional events are probably required at some later time, such as oncogene activation or viral infection [20,31]. As McCann and co-workers have suggested, the hypothesis of viral transfection of donor cells would be supported by finding the HTLV-I virus in donor cells after BMT for the T-cell leukemia associated with that virus [31], but this circumstance has not been reported.

Impaired immune surveillance: Impairment of immune surveillance due to steroid, cyclosporine, or other treatment after BMT may be a risk factor for DCL [20,25,33]. It is known that T-cell impairment by depletion of these cells from the engrafted marrow or by anti-T cell prophylaxis or treatment of GVHD after BMT predisposes the recipient to Epstein-Barr virus-associated immunoblastic lymphoma in donor cells [51].

Drug therapy: Apart from the possible role of immunosuppressive drugs, it is unlikely that drug therapy has played a role in DCL. Most patients received methotrexate for GVHD prophylaxis for limited periods after BMT. Methotrexate is not considered to be carcinogenic [52–54], although reduced folic acid intake has been related to an increased incidence of one type of malignancy [55].

Incorrect identification of origin of leukemic cells: The conclusion that recurrent leukemia involves donor cells may be incorrect, as discussed above, particularly if only cytogenetic studies have been used to identify the cell of origin.

Other possibilities: It has been suggested that donor lymphoid cells may transform as a result of antigenic stimulation of a susceptible clone [13,25] or because some homeostatic mechanism or characteristic of the microenvironment in the host, that regulates cell proliferation and maturation, has gone awry [13,21,25,33].

In conclusion, it is clear from review of the literature that proof of the cell of origin in leukemia arising after BMT may not be simple and that cytogenetic studies alone may be misleading. Molecular studies are the best means currently available to identify donor cell origin of such leukemias. The hypotheses, or speculations, put forward to explain DCL have not been proven in individual cases, and full understanding of the mechanism of this interesting disorder awaits further insights.

ACKNOWLEDGMENTS

The authors acknowledge the fellows, residents, and medical students who participated in the care of this patient over the 15 years of his illness.

REFERENCES

1. Deeg HJ, Sanders J, Martin P, Fefer A, Neiman P, Singer J, Storb R, Thomas ED. Secondary malignancies after marrow transplantation. Exp Hematol 1984;12:660–666.

2. Witherspoon RP, Fisher LD, Schoch G, Martin P, Sullivan KM, Sanders J, Deeg J, Doney K, Thomas D, Storb R, Thomas ED. Secondary cancers after bone marrow transplantation for leukemia or aplastic anemia. New Eng J Med 1989;321:784–789.
3. Lowsky R, Lipton J, Fyles G, Minden M, Meharchand J, Tejpar I, Atkins H, Sutcliffe S, Messner H. Secondary malignancies after bone marrow transplantation in adults. J Clin Oncol 1994;12:2187–2192.
4. Witherspoon RP, Deeg HJ, Storb R. Secondary malignancies after marrow transplantation for leukemia or aplastic anemia. Transplantation 1994;7:1413–1418.
5. Bhatia S, Ramsay NKC, Steinbuch M, Dusenberg KE, Shapiro RS, Weisdorf DJ, Robeson LL, Miller JS, Negla JP. Malignant neoplasms following bone marrow transplantation. Blood 1996;87:3633–3639.
6. Curtis RE, Rowlings PA, Deeg J, Shriner DA, Socie G, Travis LB, Horowitz MM, Witherspoon RP, Hoover RN, Sobocinski KA, Fraumeni JF, Boice JD Jr. Solid cancers after bone marrow transplantation. New Eng J Med 1997;336:897–904.
7. Duell T, van Lint MT, Ljungman P, Tichelli A, Socie G, Apperley JF, Weiss M, Cohen A, Nekolla E, Kolb, H-J. Health and functional status of long-term survivors of bone marrow transplantation. Ann Intern Med 1997;126:184–192.
8. Deeg JH. Delayed complications after bone marrow transplantation. In: Forman SJ, Blume KG, Thomas ED, editors. Bone marrow transplantation. Boston: Blackwell Scientific Publications; 1994. Chap 39, p 538.
9. Giralt SA, Champlin RE. Leukemia relapse after allogeneic bone marrow transplantation: a review. Blood 1994;84:3603–3612.
10. Boyd CN, Ramberg RC, Thomas ED. The incidence of recurrence of leukemia in donor cells after allogeneic bone marrow transplantation. Leuk Res 1982;6:833–837.
11. ISCN 1995: International System for Human Cytogenetic Nomenclature. Mitelan F, editor. Basel: S. Karger; 1995.
12. Hecht JT, Hogue D, Strong LC, Hansen MF, Blanton SH, Wagner M. Hereditary multiple exostosis and chrondrosarcoma: linkage to chromosome 11 and loss of heterozygosity for EXT-linked markers on chromosomes 11 and 8. Am J Hum Genet 1995;56:1125–1131.
13. Fialkow PJ, Thomas ED, Bryant JI, Neiman PE. Leukaemic transformation of engrafted human marrow cells in vivo. Lancet 1971;1:251–255.
14. Groupe Francais de Cytogenetique Hematologique. Acute myelogenous leukemia with an 8;21 translocation. A report on 148 cases from the Groupe Francais de Cytogenetique Hematologique. Cancer Genet Cytogenet 1990;44:169–179.
15. Thomas ED, Bryant JI, Buckner CD, Clift RA, Fefer A, Johnson FL, Neiman P, Ramberg RE, Storb R. Leukaemic transformation of engrafted human marrow cells in vivo. Lancet 1972;1:1310–1313.
16. Goh, K-O, Klemperer MR. In vivo leukemic transformation: cytogenetic evidence of in vivo leukemic transformation of engrafted marrow cells. Am J Hematol 1977;2:283–290.
17. Elfenbein GJ, Brogaonkar DS, Bias WB, Burns WH, Saral R, Sensenbrenner LL, Tutschka PJ, Zaczek BS, Zander AR, Epstein RB, Rowley JD, Santos GW. Cytogenetic evidence for recurrence of acute myelogenous leukemia after allogeneic bone marrow transplantation in donor hematopoietic cells. Blood 1978;52:627–635.
18. Newburger PE, Latt SA, Pesando JM, Gustashaw K, Powers M, Chaganti RSK, O'Reilly RJ. Leukemia relapse in donor cells after allogeneic bone-marrow transplantation. New Eng J Med 1981;304:712–714.
19. Marmont A, Frassoni F, Bacigalupo A, Podesta M, Piaggio G, Van Lint MS, Caimo A, de Filippi S. Recurrence of Ph^1-positive leukemia in donor cells after marrow transplantation for chronic granulocytic leukemia. New Eng J Med 1984;310:903–906.
20. Witherspoon RP, Schubach W, Neiman P, Martin P, Thomas ED. Donor cell leukemia developing six years after marrow grafting for acute leukemia. Blood 1985;65:1172–1174.
21. Smith JL, Heerema NA, Provisor AJ. Leukaemic transformation of engrafted bone marrow cells. Brit J Haematol 1985;60:415–422.
22. Palka G, Di Bartolomeo P, Iacone A, Di Girolamo G, Betti S, Calabrese G, Natale D, Stuppia L, Rieve AK, Torlontano G. Cytogenetics and bone marrow transplantation. Cancer Genet Cytogenet 1986;21: 147–157.
23. Zaccaria A, Rosti G, Testoni N, Arnold R, Bandini G, Becher R, Boogaerts M, Carbonell F, Di Bartolomeo P, Gerrant A, Filippetti A, Frassoni F, Gahrton G, Haas OA, Mecucci C, Michaux J-L, Palka G, Pollak C, Ricci P, Rosetti A, Sessarego M, Valenti S, Van den Berghe H, Tura, S. Chromosome studies in patients with acute nonlymphocytic or acute lymphocytic leukemia submitted to bone marrow transplantation—results of a European cooperative study. Cancer Genet Cytogenet 1987;26:51–58.
24. Schmitz N, Johanason W, Schmidt G, von der Helm K, Loffler H. Recurrence of acute lymphoblastic leukemia in donor cells after allogeneic marrow transplantation associated with a deletion of the long arm of chromosome 6. Blood 1987;70:1099–1104.
25. Feig SA, Dreazen O, Simon M, Wiley F, Schreck R, Gale RP. B cell acute lymphoblastic leukemia (ALL) in donor cells following bone marrow transplantation for T cell ALL. Bone Marrow Transplantation 1988;3:331–337.
26. Palka G, Calabrese G, Di Girolamo G, Stuppia L, Di Barolomeo P, Franchi PG, Di Birgilio C, Bianchi GP, Angrilli F, Parruti, G, Torlontano G. Cytogenetic survey of 31 patients treated with bone marrow transplantation for acute nonlymphocytic and acute lymphoblastic leukemias. Caner Genet Cytogenet 1991;51:223–233.
27. Browne PV, Lawler M, Humphries PM, McCann SR. Donor-cell leukemia after bone marrow transplantation for severe aplastic anemia. New Eng J Med 1991;325:710–713.
28. Cullis JO, Marks DI, Schwarer AP, Barrett AJ, Hows JM, Swirsky DM, Goldman JM. Relapse into blast crisis following bone marrow transplantation for chronic phase chronic myeloid leukaemia: a report of five cases. Brit J Haematol 1992;81:378–382.
29. McCann SR, Lawler M, Humphries P, Bacigalupo A, Podesta M, Frassoni F, Van Lint MT, Marmont A. Recurrence of Philadelphia chromosome-positive leukemia in donor cells after marrow transplantation for chronic granulocytic leukemia: confirmation by microsatellite studies (letter). Blood 1992;79:2803–2805.
30. Katz F, Reeves BR, Alexander S, Kearney L, Chessells J. Leukaemia arising in donor cells following allogeneic bone marrow transplantation for thalassaemia demonstrated by immunological, DNA and molecular cytogenetic analysis. Brit J Haematol 1993;85:326–331.
31. McCann, Lawler M, Bacigalupo A. Recurrence of Philadelphia chromosome-positive leukemia in donor cells after bone marrow transplantation for chronic granulocytic leukemia. Leukemia Lymphoma 1993; 10:419–425.
32. Mouratidou M, Sotiropoulos D, Deremitzaki K, Spathas DH, Hoffbrand AV, Prentice HG, Papanastasiou K, Tsakanikas S, Tsaftaridis P, Stamatelou M, Maniatis A. Recurrence of acute leukemia in donor cells after bone marrow transplantation: documentation by in situ DNA hybridization. Bone Marrow Transplant 1993;12:77–80.
33. Cransac M, Boiron J-M, Merel P, Cony-Makhoul P, Marit G, Bernard P, Ferber J, Reiffers J. Burkitt-type acute lymphoblastic leukemia in donor cells after allogeneic bone marrow transplantation for acute nonlymphoblastic leukemia. Transplantation 1993;56:120–123.
34. Fyles GM, Minden MD, Meharchand JM, Lipton JH, Atkins HL, Tejpar I, Messner HA. Donor cell derived acute myelogenous leukemia (AML) developing post allogeneic bone marrow transplant (BMT) for a patient with chronic myelogenous leukemia (CML). J Cell Biochem 1994;18B(suppl):63 (abstr).
35. Lowsky R, Fyles G, Minden M, Lipton J, Meharchand J, Tejpar I, Zipursky A, Messner H. Detection of donor cell derived acute myelogenous leukaemia in a patient transplanted for chronic myelogenous leukaemia using fluorescence in situ hybridization. Brit J Haematol 1996;93:163–165.

36. Anastasi J, Thangavelu M, Vardiman JW, Hooberman AL, Bian ML, Larson RA, LeBeau MM. Interphase cytogenetic analysis detects minimal residual disease in a case of acute lymphocytic leukemia and resolves the question of origin of relapse after allogeneic bone marrow transplantation. Blood 1991;77:1087–1091.
37. Biondi A, Norman C, Messner HA, Minden MD. Restriction fragment length polymorphism analysis of hematopoietic cells following successful treatment of relapsed acute lymphoblastic leukemia following bone marrow transplantation. Bone Marrow Transplant 1989;4:705–709.
38. Perla G, Bodenizza C, Falcone A, Melillo L, Scolaro E, Carotenuto M, Musto P. Misleading cytogenetic evidence of relapse in donor cells after allogeneic bone marrow transplantation corrected by fluorescent in situ hybridization. Haematologica (Pavia) 1995;80:292–293.
39. Stein J, Zimmerman PA, Kochera M, Strandjord S, Golden W, Simon M, Warkentin P, Blazar BR, Coccia P, Lang-Unnasch N. Origin of leukemic relapse after bone marrow transplantation: comparison of cytogenetic and molecular analyses. Blood 1989; 73:2033–2040.
40. Naoe T, Kiyoi H, Yamanaka K, Naito K, Yamada K. A case of cALL relapse after allogeneic BMT: recurrence of recipient cell origin, initially determined as being that of donor cell origin by sex chromosome analysis. Brit J Haematol 1989;73:420–422.
41. Chalmers EA, Sproul AM, Mills KI, Burnett AK. Cytogenetic and molecular analysis of relapse following bone marrow transplantation (letter). Br J Haematol 1990;75:631–632.
42. Minden MD, Messner HA, Belch A. Origin of leukemic relapse after bone marrow transplantation detected by restriction fragment length polymorphism. J Clin Invest 1985;75:91–93.
43. Blazar BR, Orr HT, Arthur DC, Kersey JH, Filipovich, AH. Restriction fragment length polymorphisms as markers of engraftment in allogeneic marrow transplantation. Blood 1985;66:1436–1444.
44. Ginsburg D, Antin JH, Smith BR, Orkin SH, Rappeport JM. Origin of cell populations after bone marrow transplantation. Analysis using DNA sequence polymorphisms. J Clin Invest 1985;75:596–603.
45. Knowlton RG, Brown VA, Braman JC, Barker D, Schumm JW, Murray C, Takvorian T, Ritz J, Donis-Keller H. Use of highly polymorphic probes for genotypic analysis following bone marrow transplantation. Blood 1986;68:378–385.
46. Yam PY, Petz LD, Knowlton RG, Wallace RB, Stock AD, de Lange G, Brown VA, Donis-Keller H, Blume KG. Use of DNA restriction fragment length polymorphisms to document marrow engraftment and mixed hematopoietic chimerism following bone marrow transplantation. Transplantation 1987;43:399–407.
47. Ganesan TS, Min GL, Goldman JM, Young BD. Molecular analysis of relapse in chronic myeloid leukemia after allogeneic bone marrow transplantation. Blood 1987;70:873–876.
48. Min GS, Hibbin J, Arthur C, Apperley J, Jeffreys A, Goldman J. Use of minisatellite DNA probes for recognition and characterization of relapse after allogeneic bone marrow transplantation. Br J Haematol 1988;68:195–201.
49. Fyles GM, Messner HA, Lockwood G, Curtis JE, Rider W, Minden MD, Meharchand JM, Lipton J, Tritchler D, Van Dyk J, Keane T, Sutcliffe S. Long-term results of bone marrow transplantation for patients with AML, ALL and CML prepared with single dose total body irradiation of 500 cGy delivered with a high dose rate. Bone Marrow Transplant 1991;8:453–463.
50. Niederwieser DW, Appelbaum FR, Gastl G, Gersdorf E, Meister B, Geissler D, Tratkiewicz JA, Thaler J, Huber C. Inadvertent transmission of a donor's acute myeloid leukemia in bone marrow transplantation for chronic myelocytic leukemia. New Eng J Med 1990;322: 1794–1796.
51. Deeg HJ, Socie G. Malignancies after hematopoietic stem cell transplantation: many questions, some answers. Blood 1998;91:1833–1844.
52. Rustin GJS, Rustin F, Dent J, Booth M, Salt S, Bagshawe KD. No increase in second tumors after cytotoxic chemotherapy for gestational trophoblastic tumors. New Eng J Med 1983;308:473–476.
53. Bertino JR, Romanini A. Chemotherapeutic agents. In: Holland JF, Frei E III, Bast RC Jr, Kufe DW, Morton DL, Weichselbaum RR, editors. Cancer medicine, 3rd edition. Philadelphia: Lea & Febiger; 1993. Chap 3, p 698.
54. Moder KG, Tefferi A, Cohen MD, Menke DM, Luthra HS. Hematologic malignancies and the use of methotrexate in rheumatoid arthritis: a retrospective study. Am J Med 1995;99:276–281.
55. Giovannucci E, Stampfer MJ, Colditz GA, Hunter DJ, Fuchs C, Rosner BA, Speizer FE, Willett WC. Multivitamin use, folate, and colon cancer for women in the Nurses' Health Study. Ann Intern Med 1998; 129:517–524.